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He-Ne Laser Induced Germination of Endospores of *Anaerobacter polyendosporus*

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The action of He-Ne laser (λ = 632.8 nm) radiation upon germination of endospores of anaerobic Gram positive bacteria *Anaerobacter polyendosporus* was studied. As compared with the nonirradiated control (100%), the percentage of germinated spores was higher when irradiated in the dose range from 150 to 450 J/m^2 with a maximum (150%) at a dose of 300 J/m^2.

KEYWORDS: *He-Ne laser, germination, endospore,* Anaerobacter polyendosporus (Bacillus.)

INTRODUCTION

Irradiation with various bands of visible light including red, was found to increase the division rate of vegetative cells of microorganisms, e.g. bacteria *Escherichia coli* [1, 2] and various yeasts [3, 4].

In addition to the state of the vegetative growth, the natural life cycle of many microorganisms include a resting or dormant state (spore) [5]. Spores are characterized by great resistance to heat, desiccation, pressure, vacuum, UV and ionizing radiation, chemicals and extremes of pH[6].

As an example, Figure 1 presents the scheme of the life cycle of prokaryotic microorganisms forming endospores (e.g., genera *Bacillus, Clostridium, Sporosacrina, Thermoactinomyces).* These endospores are fundamentally different from other types of spores because they are formed within the vegetative cells, none of the cell walls (cortex) being derived from the wall of the mother cell [5].

The present work is an investigation of the possibility of increasing bacterial endospore germination by irradiation with He-Ne laser. Germination is a well-defined stage in the developmental cycle of spore-forming bacteria and means the conversion of a resistant and dormant spore into a sensitive and metabolically active form [6]. Fresh endospores exposed optimal germination conditions will not germinate in most cases unless conditioned (activated) to germination. Activation itself does not initiate the germination, but afterwards allows spores to germinate more rapidly, more completely or both [7]. The phenomenon of

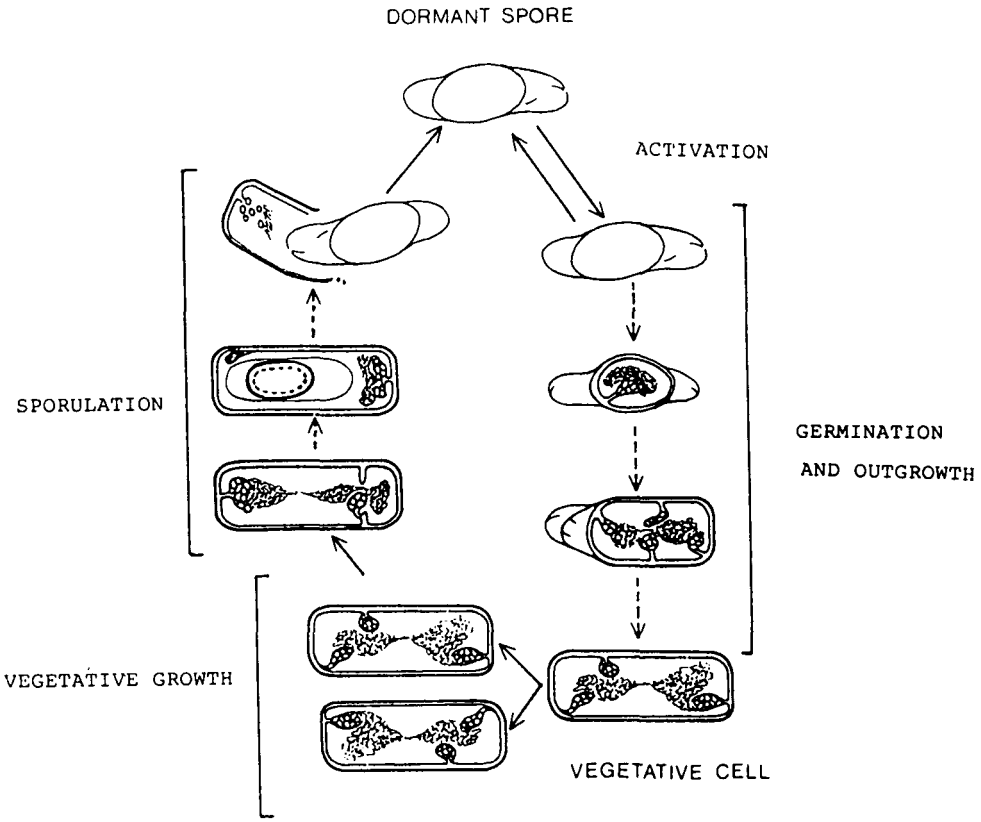

FIGURE 1 Scheme of the life cycle of the bacteria forming endospores.

activation has been observed only for endospores and not for other types of resting cells [5]. Activation does not involve metabolic processes and consists apparently of changes in the configuration of macromoles [5, 7]. Its mechanism is not understood. There is probably more than one reaction mechanism because of the existence of a great number of methods of activation (exposure tosublethal heat, low pH, thiol compounds and strong oxidizing agents [7]). It is known that filtered sunlight at wavelengths 360-380 nm induces *Bacillus thuringiesis* spore germination even in the presence of germination inhibitors [8]. In the literature available to us we did not find the data about visible laser light-induced spore germination.

These experiments are also a part of experimental work performed with prokaryotic andeukaryotic cells for explaining the molecular mechanism of low-power laser therapy [for review, see9, 10].

MATEMALS AND METHODS

Bacterial strain. The general characteristics of *Anaerobacter polyendosporus* (genus *Bacillus)* used in this study have been described elsewhere [II]. This is a Gram positive

facultative anaerobic strain separated from soil of Birmean rice fields. From a microbiological point of view, it is interesting to note that by sporulation one cell of this strain forms 3 to 5 endospores [II].

Chemicals. All chemicals were reagent grade and purchased from Reanal (Budapest) or Chemapol (Prague).

Medium and growth conditions. The culture was grown on a mineral-agar medium with a composition according to Pfenning and Lippert [12]: 0.33 g/1 K_2PO_4, NH_4Cl, $MgCl_2 \cdot H_2O$, $CaCl_2 \cdot 6H_2O$, KC1; 15 g/1 $NaHCO_3$; 1 ml microelements, 20 g/1 agar and 1 g/1 glucose, pH 7.2-7.4.

Sporulation. The cells were allowed to grow and sporulate for 7-9 days in aerobic conditions at 28°C until practically all spores had been released from the sporangia. At the end of sporulation, no sporangia and no large fragments were observed with the microscope. The endospores were found to be similar with the endospores described in literature [II]. The spores were collected on Whatman No. 1 filter paper and washed repeatedly with sterile distilled water. This material was used for further experiments.

Irradiation. The spores were suspended in distilled water in a concentration of 4×10^8 cells/ml. This concentration was found earlier to be optimal for irradiation of vegetative cells [3, 4]. Five ml of the suspension was irradiated in a round glass flask with a 75 mm bottom diameter as described in Fedoseyeva *et al.* [3]. A special objective was used for homogeneous irradiation of the whole area. For irradiation, a He-Ne laser (LG-75, Lvov, USSR, $\lambda = 632.8$ nm, $\lambda = 4.4$ W/m^2 on the level of suspension) was used. Irradiation was performed in the dark. Control samples were maintained under the same conditions without irradiation.

Spore germination. Just after irradiation, 0.02 ml of irradiated or intact suspension was plated on Petri dishes with Pfenning growth medium and put into a special vacuum flasks from which the air was then removed with a vacuum pump. The number of outgrown spores was determined by counting the colonies after 5-6 days of cultivation at 28°C.

Nine series of experiments were run, each involving six duplicates.

RESULTS AND DISCUSSION

In our experiments 2×10^6 endospores were plated on every Petri dish. The number of germinated and outgrown endospores (counted as a number of formed colonies) was 300 ± 25 (0.015%).

The effect of irradiation manifested itself in the increase of the number of germinated and outgrown spores. As seen in Figure 2, the number of outgrown spores depends on the irradiation dose, starting to increase at doses higher than 150 J/m^2 and being maximal (1.5 times higher than in intact control) at 300 J/m^2. Further increase in the dose caused a decrease of the number of outgrown spores, being on the control level at the dose near 450 J/m^2.

Thus, it was found that the irradiation with He-Ne laser at particular doses increased the number of germinated and outgrown spores. It is interesting to remember that this kind of bell-shaped dose dependency is characteristic of various types of visible light effects [9,10].

Low-power laser effects are considered to be photobiological phenomena [9]: the cascade of reactions starts from absorption of light in the mitochondria and after that the signal is transduced to the cellular membrane and from the membrane to the nucleus [10]. Whether this is the case for germination is not clear. First, an action spectrum reflecting the absorption

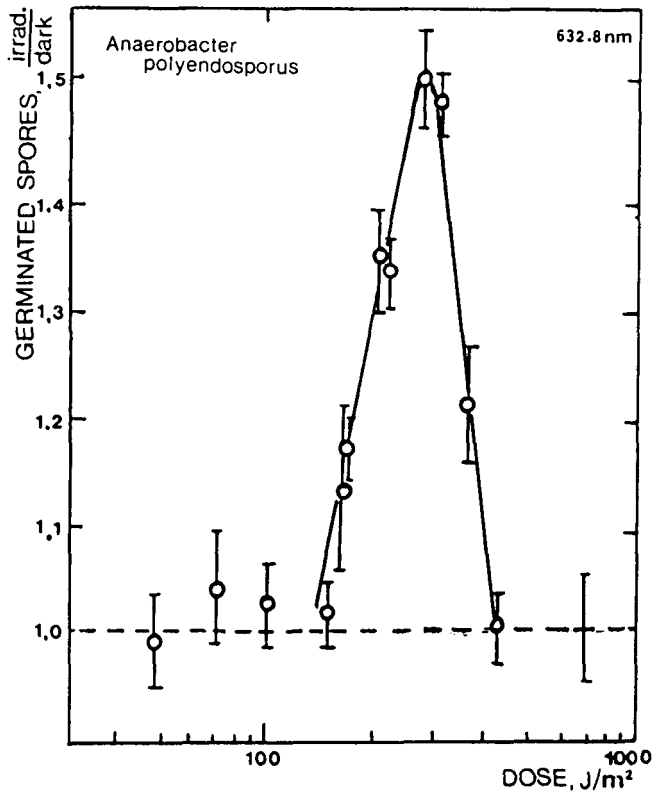

FIGURE 2 Percentage of germinated and outgrown endospores after the irradiation of *Anaerobacter polyendosporus* endospores with He-Ne laser at different doses.

spectrum of primary photoacceptor(s) is absent. Second, as distinct from the vegetative cells, the spores are dormant cells in which the respiration is hardly detectable[13]. The dormant endospores contain approximately 5% of the amount of components of respiratory chain as compared with vegetative cells [14-16]. Almost complete absence of ATP (only —0.40% of this in vegetative cells) in endospores is consistent with the absence of endogenous metabolism and macromolecular biosynthesis [16].

The components of respiratory chain (cytochromes and flavoproteins) by their spectro-scopic characteristics can absorb the light at λ= 632.8 nm and work as primary photoacceptors, as was proposed for vegetative cells [9, 10]. Data from this paper do not support the conclusion that the mechanism of light stimulation is the same in both cases. It should also be remembered that the molecular mechanism of germination is not fully understood[5-7]. In favor of the light action upon respiratory chain components is the finding that, as a rule, the first 5 min of germination are accompanied by an abrupt increase in ATP content[16]. Second, some chemical compounds which activate the germination were found to stimulate the activity of endogenous electron transport systems in spores [7]. Association of this process with He-Ne laser radiation induced germination needs further clarification. At the moment, we can only conclude that the irradiation with light atλ = 632.8 nm at dose range from 150 to 450 J/m^2 increases the number of germinated and outgrown endospores.

References

1. Tiphlova,0. A. and Karu, T. I.: *Escherichia coli* growth stimulation by low intensity monochromatic visible light. *Photochem. Photobiol.* **47**:467-473, 1988.
2. Tiphlova, 0. A. and Karu, T. I.: Role of primary photoacceptors in low-power laser effects: action of He-Ne laser radiation on the bacteriophage *T4-Escherichia coli* interaction. *Lasers Surg. Med.* **9**:67-69, 1989.
3. Fedoseyeva,G.E.,Karu,T.I.,Lyapunova,T.S.,Pomoshnikova,N.A.,andMeissel,M.N.:Theactivationof yeast metabolism with He-Ne laser radiation. I. Protein synthesis in various cultures. *Lasers Life Sci.* **2**:137-146, 1988.
4. Fedoseyeva,G.E.,Karu,T.I.,Lyapunova,T. S., Pomoshnikova, N. A., andMeissel.M. N.:The activation of yeast metabolism with He-Ne laser radiation. II. Activity of enzymes ofoxidative and phosphorous metabolism. *Lasers Life Sci.* **2**:147-154, 1988.
5. Sudo, S. Z. and Deworkin, M.: Comparative biology ofprokaryotic resting cells. *laAdvs. Microbiol. Physiol.,* A. H. Rose and D. W. Tempest (eds.). New York, London: Academic Press, Vol. **9**, pp. 153-224.
6. Gould, G. W: Germination. In *The Bacterial Spore, G.* W. Gould and A. Hurst (eds.), London, New York: Academic Press, 1969, pp. 397-444.
7. Keynan, A., andEvenchik, Z.: Activation. *In The Bacterial Spore, G.* W. Gould and A. Hurst (eds.), London, New York: Academic Press, 1969, pp. 359-396.
8. Hoffman, S. B., Travers, R. S., and Martin, R A.W: Light induced germination *of Bacillus thuringiensis* spores. *Abstracts of the Annual Meeting of the American Society for Microbiology,* Washington: American Society for Microbiology, 1986, p. 182. (Abstract 1-102).
9. Karu, T. I.: Photobiological fundamentals of low-power laser therapy. *IEEE J. Quant. Electronics* QE-**23**:1703-1717, 1987.
10. Karu, T. I.: *Photobiology of Low-Power Laser Therapy,* New York: Harwood Academic Publishers, 1989.
11. Duda,V. I., Mushegian, M. S., Lebedinskii, A. V, and Matyushina, L. A.: Forming of four to fiveendospores in one cell of a new anaerobic bacterial strain. *Dokl. Akad. Nauk SSSR (Proceedings of USSR Academy of Sciences, Microbiology),* **285**:241-245, 1985.
12. Pfenning, N. and Lippert, K. D.: Uber das Vitamin B,,—Bedurfnis phototropher Schwefelbakterien. *Arch. Microbiol.* **55**:245-256, 1966.
13. Darwes, I. W. and Mandelstam, J.: Sporulation *of Bacillus subtilis* in continuous cultures. *J. Bacterial.* **103**:529-535, 1970.
14. Felix, J. A. and Lundgren, D. G.: Electron transport system associated with membranes *of Bacillus cereus* during vegetative growth and Sporulation. *J. Bacterial.* **115**:552-559, 1973.
15. Weber, M. M., and Broadbent: Electron transport in membranes from spores and from vegetative and mother cells of *Bacillus subtilis.* In *Spores VI,* R Gerhard, R. N. Castilow, and H. Sadoff (eds.), Washington:American Society of Microbiology, 1975, pp. 411-417.
16. Setlow, R: Energy and small-molecules metabolism during germination of *Bacillus* spores. In *Spores VI,* P Gerhardt, R. N. Costilow, and H. Sadoff (eds.), Washington: American Society of Microbiology, 1975, pp. 443-450.